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Journal

Biogeographia – The Journal of Integrative Biogeography, 34(0)

ISSN

1594-7629

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Publication Date

2019

DOI

10.21426/B634043868

On the non-malacostracan crustaceans (Crustacea: Branchiopoda, Copepoda, Ostracoda) from the inland waters of Fthiotida (Greece)

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Keywords: *Arctodiaptomus alpinus*, Cross-taxon congruence, *Diaptomus* cf. *serbicus*, *Leptestheria dahalacensis*, Temporary ponds, *Wlassicsia pannonica*.

SUMMARY

In the frame of the activities of the LIFE11 NAT/GR/1014 ForOpenForests, some water bodies occurring in the "Ethnikos Drymos Oitis" (GR2440004) and "Oros Kallidromo" (GR2440006) (Sterea Ellada) were investigated with the aim of providing a first census of the composition and diversity of their crustacean fauna. Overall, the sampling of 15 water bodies (7 of them listed as "Mediterranean temporary ponds" *sensu* EU "Habitats Directive") led to the finding of 13 branchiopod, 11 copepod, and 7 ostracod taxa, including 4 species new for mainland Greece, i.e. the copepods *Arctodiaptomus alpinus* (Imhoff, 1885) and *Diaptomus* cf. *serbicus*, and the branchiopods *Leptestheria dahalacensis* (Rüppel, 1837) and *Wlassicsia pannonica* Daday, 1904. The comparative analysis of the observed species assemblages and richness suggests that the protection of those ponds identified as "priority habitats" according to the "Habitats Directive" is effective for the specialized and peculiar crustacean biota of these ecosystems, but it is not sufficient in order to preserve efficiently the whole diversity of temporary pond-dwelling crustaceans occurring in the study area. Therefore, the implementation of synergistic conservation measures dedicated to both "priority" and "non-priority" habitats is desirable.

INTRODUCTION

Non-malacostracan, free-living crustaceans are among the most abundant and diverse invertebrate taxa inhabiting temporary ponds (e.g., Williams 2005). Thanks to the production of drought-resistant resting stages, these crustaceans can survive through the dry phases periodically experienced by these unstable ecosystems (Incagnone et al. 2015).

Due to their unusual aspect, high rate of endemism, and keystone ecosystem role, the so-called ‘large branchiopods’ (Branchiopoda: Anostraca, Notostraca, Spinicaudata) and the calanoid copepods (Copepoda: Calanoida) are considered “flagship taxa” of the animal biota inhabiting Mediterranean temporary ponds (e.g., Sahuquillo and Miracle 2013, Alfonso et al. 2016, Marrone et al. 2016). Notwithstanding the paramount importance of crustaceans in pond ecosystems, to date only few data are available about the non-malacostracan crustaceans inhabiting the inland waters of Greece (see Abatzopoulos et al. 1999, Marrone 2006, Marrone et al. 2019, and references therein); such lack of data is particularly regrettable since, due to its geographical location and complex physiography, Greece is expected to host an extraordinarily rich and diverse crustacean fauna (e.g., Griffiths et al. 2004, Blondel et al. 2010, Marrone et al. 2017).

Within the frame of the activities of the LIFE11 NAT/GR/1014 ‘ForOpenForests’ program, we investigated the crustacean fauna of the temporary water bodies occurring in the Natura 2000 sites "Ethnikos Drymos Oitis” (GR2440004) and “Oros Kallidromo” (GR2440006), located in the eastern Aegean (Attiko-Voiotia) Greek ecoregional unit, as defined by Zogaris et al. (2009. Moreover, a comparison between the crustacean assemblages inhabiting the habitat of Community Interest 3170* (Mediterranean temporary ponds, listed in Annex 1 of the Habitats Directive 92/43/EEC) and those observed in other temporary and permanent water bodies occurring in the study area was carried out.

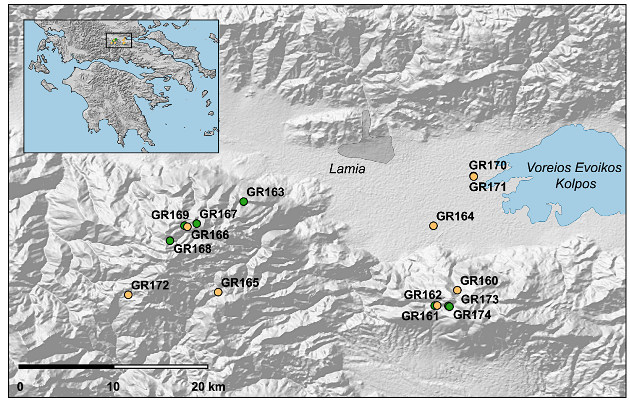

Figure 1. Geographical location of the sampled sites (Sterea Ellada, Greece). 3170* sites are reported in green, “non-3170*” sites are reported in yellow.

MATERIALS AND METHODS

Samples were collected from 13 temporary and two permanent water bodies in the study area on May and November 2017 (see Fig. 1, Table 1, Appendix 1); the geographical coordinates and elevation of each site were recorded using a hand-held GPS apparatus, and an alphanumeric code was attributed to each of them (Table 1). The locality names and any other information originating from the Greek language follow the transliteration rules as set by the ELOT 743/ ISO 843 system, to properly standardize the wording. This should be an integral part of all such reports, as it streamlines the proper databasing of specimens and avoids data-mining misinterpretations based on misspellings (see above example of Ethnikos Drymos Oitis, which could be erroneously inscribed as Ethnikos Drimos Itis).

Seven out of the 15 sampled water bodies could be ascribed to the priority habitat type “Mediterranean temporary ponds (3170*)”; these are freshwater temporary ponds located between 989 and 1914 m a.s.l. The ponds on Oiti are characterized by a somewhat regular alternation between their wet and dry phases, while those on Kallidromo are rather irregular, with the wet phase not being in sync between them (Delipetrou et al. 2015a). The main typical flora species in the water bodies of Oiti are annuals such as *Lythrum thymifolia* L., *Limosella aquatic* L., *Ranunculus lateriflorus* DC, *Myosurus minimus* L., and *Veronica oetaea* L. -A. Gustavsson 1978, with the latter being a local endemic and registered as a priority species in Annex II of the EU92/43 Directive (Karetsos et al. 2018, Delipetrou et al. 2015b). For the Kallidromo ponds, typical species are *Verbena supina* L., *Heliotropium supinum* L., *Mentha pulegium* L., and *Cyperus fuscus* L.. The vegetation shows temporal and spatial variation, depending mainly on water depth and inundation period (Delipetrou et al. 2015a, Karetsos 2002). As for the fauna, Legakis (2015) recorded larval forms of insects (e.g., Odonata, Trichoptera, Ephemeroptera, Heteroptera), predatory adult beetles (Coleoptera; probably Hydrophilidae and Gyrinidae), as well as Platyhelminthes and Annelida. Six other water bodies, located along an elevational range comprised between 1 and 1536 m a.s.l., were characterized by a temporary hydroperiod, but did not host the phytosociological associations typical of habitat 3170*.

Water temperature (°C) and electrical conductivity (μS/cm) were measured using a hand-held Hanna Instruments HI9835 multiprobe. Three arbitrary qualitative classes were used to estimate water turbidity (from 1: crystal-clear water, to 3: extremely turbid water). Macrophyte cover was estimated visually observing the pond surface; the percentage of macrophyte cover was used as a proxy for the structural complexity of aquatic vegetation and hence, habitat complexity, varying from 0 (low complexity, macrophytes absent) to 3 (high complexity, 100% macrophyte cover).

A 200μm mesh-sized hand net was used to sample crustaceans along shorelines and through submerged vegetation; a 125μm mesh-sized towing net was used to collect samples in the open waters. Collected samples were fixed *in situ* in 96% ethanol.

Table 1. List of the sampled sites. Geographical coordinates are in decimal degrees, WGS84 datum.

Sites	Mountain body	ID Code	Latitude	Longitude	Elevation (m a.s.l.)	Hydroperiod	Habitat 3170*
Limni Nevropolis	Kallidromo	GR161	38.751484	22.492133	989	Temporary	YES
Limni Louka	Oiti	GR163	38.843127	22.323721	1159	Temporary	YES
Limni Greveno	Oiti	GR167	38.823732	22.282342	1893	Temporary	YES
Limni Alikaina	Oiti	GR168	38.808661	22.258798	1914	Temporary	YES
Limni Livadies	Oiti	GR169	38.821925	22.271584	1813	Temporary	YES
Mourouza	Kallidromo	GR173	38.751262	22.504197	1075	Temporary	YES
Mourouzos	Kallidromo	GR174	38.750576	22.505173	1075	Temporary	YES
Pond 1 Anopia	Kallidromo	GR160	38.765111	22.512111	906	Temporary	NO
Nevropoli pool	Kallidromo	GR162	38.751625	22.494335	989	Temporary	NO
Anthili rice fields	-	GR164	38.821856	22.491015	5	Temporary	NO
Pond 1 Katavothra	Oiti	GR165	38.763363	22.301297	1536	Temporary	NO
Pond at the mouth of Spercheios river	-	GR170	38.865764	22.526366	1	Temporary	NO
Pond close to the village of Kastriotissa	Oiti	GR172	38.761030	22.222049	1400	Temporary	NO
Man-made pond on the path to Livadies	Oiti	GR166	38.820980	22.274155	1808	Permanent	NO
Reservoir at the mouth of the Spercheios river	-	GR171	38.865176	22.526493	1	Permanent	NO

The *ex situ* re-hydration of dry sediment, known as "Sars' method" (van Damme and Dumont 2010) was used as a complement to the study of the crustacean samples collected during the inundated phase of temporary water bodies. Accordingly, sediment samples were collected from some of the sampled water bodies (i.e GR164, GR168, GR173, GR174, see Table 1) and cultured in laboratory following the methods described by Marrone et al. (2019).

Crustacean specimens collected in the field or coming from laboratory cultures were sorted and identified according to Cottarelli and Mura (1983), Alonso (1996), Flössner (2000) and Korn et al. (2006) (Branchiopoda), Dussart (1967, 1969) and Kiefer (1978) (Copepoda), and Meisch (2000) (Ostracoda); identifications and nomenclature were updated following the most recent taxonomic literature. Undissected crustacean specimens were stored in 95% ethanol at -20°C; dissected soft parts were mounted in glycerol in sealed microscope slides.

Because of the intricate taxonomy of the anostracan genus *Chirocephalus* and, in particular, of the species included in the *C. diaphanus* group, five specimens of *C. diaphanus sensu lato* from sites GR161, GR163, GR167, GR169 and GR174 (see Table 1) were studied by amplifying and sequencing a fragment of the mitochondrial gene encoding for the cytochrome oxidase subunit I (COI); comparative sequences of *C. diaphanus* s.l. were downloaded from GenBank and included in the analysis (see Marrone et al. 2019, for details on the laboratory protocols and data analysis). Likewise, a fragment of the mitochondrial 12S gene was amplified for performing molecular identification of the specimens of the notostracan genus *Triops* raised from the sediment collected in the Anthili rice fields (see Tziortzis et al. 2014 for details on the laboratory protocols and data analysis). The new sequences were deposited in GenBank with the accession numbers MK748499-MK748503 (*Chirocephalus* COI sequences) and MK736275 (*Triops* 12S sequence). For each dataset, best-fitting evolutionary models were selected under the Akaike Information Criterion (AIC) with MrModeltest 2.2 (Nylander 2004). Bayesian inference (BI) and maximum likelihood (ML) analyses were performed as implemented by MrBayes 3.2 (Ronquist et al. 2012) and PhyML v.3 (Guindon and Gascuel 2003), respectively.

Data obtained from the field surveys were stored as a binary (presence/absence) species composition data matrix due to the difficulty to compare species abundances obtained using different collecting methods. Sites GR168 and GR170 were excluded from the analysis since no crustaceans were collected there, nor obtained from culturing the sediment samples. Site GR162 was also not included, since it hosts a subset of the fauna occurring in the neighbouring site GR161, which is likely the source area for a part, or the totality, of the fauna recorded in GR162 (cf. Table 2). Each sample was attributed to its habitat type defined as (i) Mediterranean temporary ponds (habitat 3170*) and (ii) non-3170 natural ponds; two water bodies were classified as rice fields ("Anthili rice fields", code GR164) and brackish water permanent ponds (reservoir located at the mouth of the Spercheios river, code GR171), respectively. All statistical analyses were performed using R software 3.3.2 (R Development Core Team 2016). A pairwise dissimilarity matrix based on the Sorensen's index of dissimilarity was computed using the R package 'vegan' (Oksanen et al. 2017). The significance of attributing sites on the basis of their dissimilarities to the two main different habitat types defined above (3170* and non-3170* ponds) was assessed by means of Analysis of Similarities (ANOSIM) using the 'vegan' R package. Ordination of sites was performed by non-Metric Multidimensional Scaling (nMDS) using the 'vegan' R package. Species were superimposed on the nMDS ordination plain using their Spearman's correlation coefficients with axes.

Table 2. Checklist and distribution of the collected non-malacostracan crustaceans.

Taxa	Acronym	Sites of occurrence (3170* only)	Sites of occurrence (non-3170*)
BRANCHIOPODA			
Anostraca			
Chirocephalidae			
Chirocephalus diaphanus Prévost, 1803	Cdia	GR161, GR163, GR167, GR169, GR173, GR174	GR162
Notostraca			
Triopsidae			
Triops cancriformis (Bosc, 1801)	Tcan		GR164
Spinicaudata			
Leptestheriidae			
Leptestheria dahalacensis (Rüppel, 1837)	Ldah		GR164
Anomopoda			
Moinidae			
Moina brachiata (Jurine, 1820)	Mbra	GR161, GR169, GR173, GR174	GR162, GR164, GR165
Moina macrocopa (Straus, 1820)	Mmac		GR164
Daphniidae			
Ceriodaphnia reticulata (Jurine, 1820)	Cret	GR169	GR166
Simocephalus vetulus (Müller, 1776)	Svet	GR161	
Daphnia (*Ctenodaphnia*) *atkinsoni* Baird, 1859	Datk	GR173, GR174	
Daphnia (*Ctenodaphnia*) *chevreuxi* Richard, 1896	Dche	GR173, GR174	
Chydoridae			
Chydorus sphaericus (Müller, 1776)	Csph	GR161	GR160, GR165, GR166, GR172
Coronatella rectangula G.O. Sars, 1862	Cret	GR161	GR166
Macrothrichidae			
Macrothrix hirsuticornis Norman & Brady, 1867	Mhir	GR161, GR169, GR174	
Wlassicsia pannonica Daday, 1904	Wpan		GR164
COPEPODA			
Calanoida			
Diaptomidae			
Arctodiaptomus alpinus (Imhoff, 1885)	Aalp	GR161, GR173, GR174	
Arctodiaptomus pectinicornis (Wierzejski, 1887)	Apec		GR165, GR166
Diaptomus cf. *serbicus* Gjorgjevic, 1907	Dser	GR173, GR174	
Pseudodiaptomidae			
Calanipeda aquaedulcis Kritschagin, 1873	Caqu		GR171
Cyclopoida			
Cyclopidae			
Eucyclops serrulatus (Fischer, 1851)	Eser	GR161	GR166, GR171
Metacyclops minutus (Claus, 1863)	Mnin	GR161, GR169, GR173, GR174	GR162
Cyclops ankyrae Mann, 1940	Cank	GR163	GR165
Diacyclops lubbocki (Brady, 1869)	Dlub		GR160
Microcyclops rubellus (Lilljeborg, 1901)	Mrub		GR160
Acanthocyclops einslei Mirabdullayev & Defaye, 2004	Aein	GR169	GR160, GR165, GR166, GR171, GR172
Harpacticoida			
Canthocamptidae			
Canthocamptus staphylinus (Jurine, 1820)	Csta		GR160
OSTRACODA			
Podocopa			
Cyprididae			
Eucypris virens (Jurine, 1820)	Evir	GR161	
Heterocypris incongruens (Ramdohr, 1808)	Hinc	GR161, GR167, GR169, GR173, GR174	GR164
Potamocypris unicaudata Schäfer, 1943	Puni	GR161	
Tonnacypris lutaria (Koch, 1838)	Tlut	GR167, GR173, GR174	
Cypria ophthalmica (Jurine, 1820)	Coph		GR166
Ilyocyprididae			
Ilyocypris decipiens Masi, 1905	Idec	GR161	
Candonidae			
Cyclocypris ovum (Jurine, 1820)	Covu		GR160, GR165, GR166, GR172

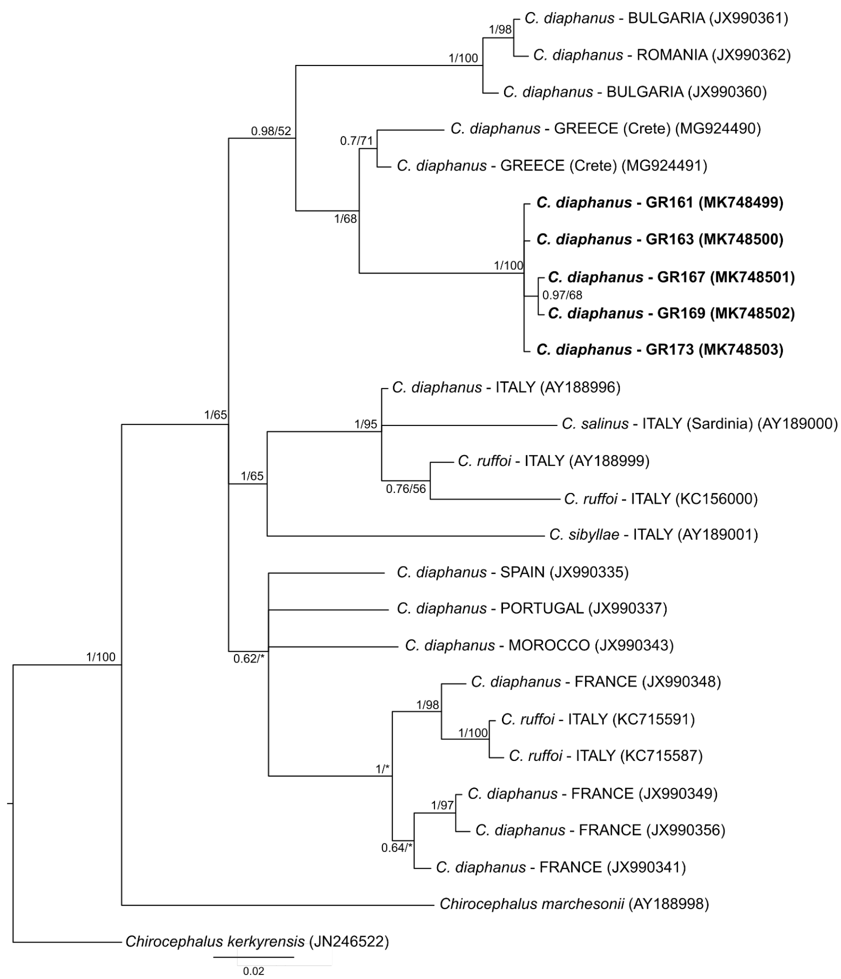

Figure 2. Bayesian consensus phylogenetic tree based on a 408bp-long fragment of mtDNA COI gene of *Chirocephalus diaphanus* s.l.. Node support is reported as nodal posterior probability/ML bootstrap; nodal posterior probability lower than 0.5 is represented as an unresolved polytomy, "*"indicates bootstrap support lower than 50. Accession numbers of sequences derived from GenBank are shown in brackets. Novel samples are reported in bold.

RESULTS

Details on the sampling sites and dates, and on the environmental parameters registered for each sampling, are reported in Appendix 1.

Overall, based on the study of both field-collected and laboratory-reared samples, 13 branchiopod, 11 copepod and 7 ostracod taxa were observed in the 15 sampled water bodies (Table 2). Oddly, no crustaceans were collected in Limni Alykaina (GR168) and in the temporary salty pond at the mouth of the Spercheios river (GR170), nor they were obtained through the culturing of the sediment.

All the collected specimens were identified at the species level; however, the diaptomid copepods belonging to the genus *Diaptomus* collected in November 2017 in Mourouza (GR173) and Mourouzos (GR174) displayed a peculiar combination of morphocharacters that deserves a more detailed analysis. Accordingly, this taxon is here provisionally reported as *Diaptomus* cf. *serbicus*.

The most frequently occurring crustacean species within the study area were the anostracan *Chirocephalus diaphanus* s.l., the ostracod *Heterocypris incongruens*, the

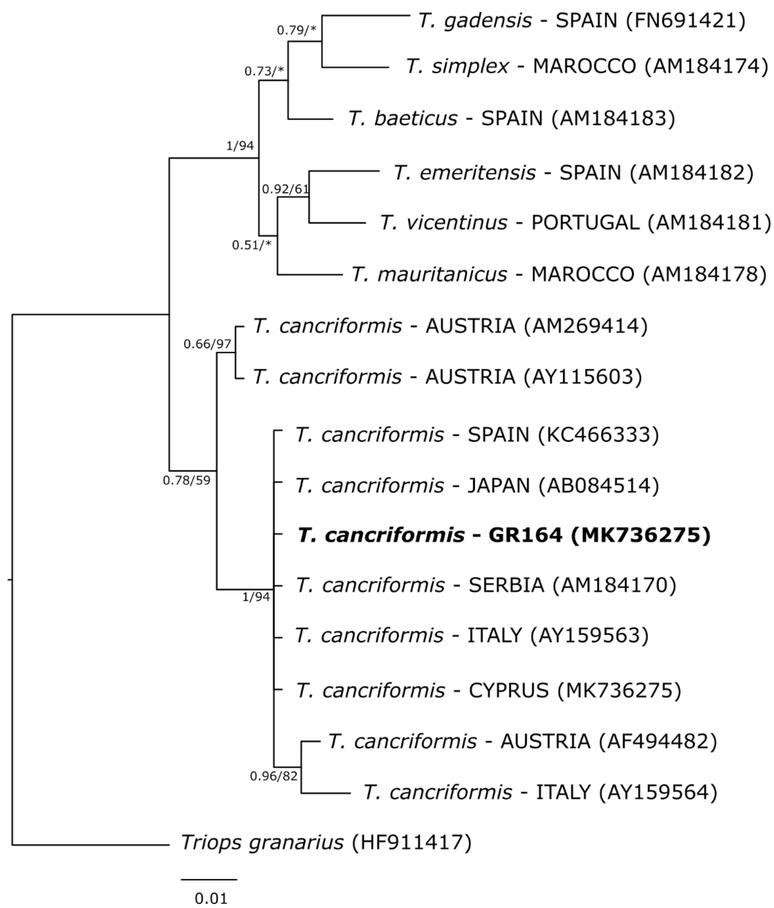

Figure 3. Bayesian consensus phylogenetic tree based on a 348bp-long fragment of the mitochondrial small subunit ribosomal DNA 12S of *Triops cancriformis*. Node support is reported as nodal posterior probability/ML bootstrap; nodal posterior probability lower than 0.5 is represented as an unresolved polytomy, "*" indicates bootstrap support lower than 50. Accession numbers of sequences derived from GenBank are shown in brackets. The novel sample is reported in bold.

cyclopoid *Acanthocyclops einslei* and the cladoceran *Moina brachiata*, recorded in six sites each. Conversely, in the frame of the present survey, several crustacean species were observed in single water bodies, thus showing a very restricted distribution in the study area.

The mtDNA sequences of the analysed *Chirocephalus diaphanus* s.l. specimens place them in a well-characterized clade that is phylogenetically close to the other Balkan populations for which molecular data are available (Fig. 2). The analysis of the mtDNA sequence of the notostracan specimen raised from sediment collected in the Anthili rice fields assigned it to a widespread lineage of *Triops cancriformis* sensu stricto (Fig. 3).

The crustacean assemblage occurring in the Mediterranean temporary ponds (3170*) was significantly different from those occurring in the other temporary ponds and in the few sampled permanent water bodies (ANOSIM analysis, Global R=0.853, P<0.01). This is clearly displayed in the nMDS plot, which showed four separate clusters (Fig. 4a) corresponding to the four different habitat types examined. The anostracan *Chirocephalus diaphanus* s.l., together with the cladocerans *Daphnia atkinsoni* and *D. chevreuxi*, the calanoids *Arctodiaptomus alpinus* and *Diaptomus* cf. *serbicus*, the cyclopoid *Metacyclops minutus*, and the ostracods *Heterocypris incongruens* and *Tonnacypris lutaria* were preferentially found in the

Mediterranean temporary ponds (Fig. 4b); *Triops cancriformis*, together with the spinicaudatan *Leptestheria dahalacensis* and the cladocerans *Moina macrocopa* and *Wlassicsia pannonica*, characterized the rice fields. The other species were found preferentially in other types of temporary and permanent ponds (see Fig. 4b), while the pseudodiaptomid calanoid *Calanipeda aquaedulcis* characterized the brackish water pond.

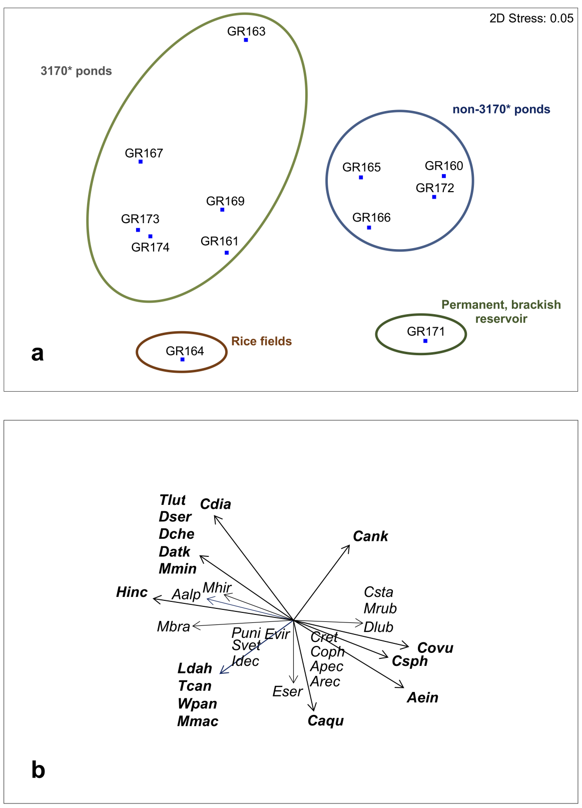

Figure 4. (a) Results of the non-metric multidimensional scaling (nMDS); ordination diagram using the first two nMDS axes (2D stress=0.05); sampling sites codes as in Table 1; ellipses delimit the habitat types (see text). (b) Ordination of crustacean species (acronyms as in Table 2) obtained by superimposing the species on the plane defined by the two nMDS axes on the basis of their Spearman's correlation (rs) with the axes: species with a significant rs are in bold; some short arrows are omitted for clarity.

DISCUSSION

Notes on the collected species

1. Branchiopoda

Overall, three large branchiopod species were collected in the frame of the present survey (Table 2). *Triops cancriformis* and *Leptestheria dahalacensis*, both collected in the Anthili rice fields, are widespread Palearctic taxa, often linked with rice fields throughout the western Palearctic. In Greece, the presence of *Triops cancriformis* was already reported for rice fields in the Axios delta (Kazantzidis and Goutner 2005), and some specimens collected in Athens are conserved in the collections of the Natural History Museum "La Specola" of the University of Florence, Italy (Innocenti 2009). The spinicaudatan *Leptestheria dahalacensis* is new for the fauna of the country and, to our knowledge, this represents the first record of the whole order Spinicaudata for Greece. Both taxa are possibly allochthonous in the study area, and the resting eggs of these species could have been unwarily introduced in the Anthili rice fields, along with rice seeds or seedlings. However, lacking detailed information on the checklist and distribution of Greek large branchiopods (see the discussion in Abatzopoulos et al. 1999), their native status cannot at present be excluded.

The molecular analysis of six *Chirocephalus diaphanus* specimens from the study sites showed the existence of a well-characterised clade nested within the "easternmost clade" of *C. diaphanus* s.l.., which should, in fact, be possibly ascribed to *C. carinatus* Daday, 1910 or *Chirocephalus romanicus* Stoicescu, 1992 (see discussion in Marrone et al. 2019) (Fig. 2). Interestingly, *C. diaphanus* s.l. was observed in all the surveyed 3170* ponds (with the obvious exception of Limni Alikaina, GR168), whilst proved to be absent in all the other surveyed water bodies with the single exception of GR162, i.e. a shallow temporary pool located a few metres apart from Limni Nevropolis. *Chirocephalus diaphanus* s.l. is a rare taxon in Greece, where it has been reported for Makedonia (Daday 1910), Voiotia (Pesta 1921), Zakynthos (Stephanides 1948), and Kriti (Marrone et al. 2019).

Among the ten collected anomopod species, the macrothrichid *Wlassicsia pannonica* is a new record for the fauna of Greece. It was collected in the Anthili rice fields (GR164), where it was possibly introduced along with the large branchiopods *Triops cancriformis* and *Leptestheria dahalacensis*. The other recorded anomopod species are widespread Palearctic taxa already known to occur in the country.

2. Copepoda

The collected harpacticoid and cyclopoid copepod species are rather widespread in the Mediterranean area; conversely, all the collected diaptomid species are of biogeographical interest. In particular, *Arctodiaptomus alpinus* is a Palearctic species, typical of high-elevation ponds (Błędzki and Rybak 2016, Marrone et al. 2017) whose presence in mainland Greece was to date unknown (see discussion in Marrone et al. 2019). *Arctodiaptomus pectinicornis* is an eastern European species reported in Greece only in two lakes close to the North Macedonia border (Kiefer 1978, Zarfdjian and Economidis 1989). To date, no information on the phenology and distribution of *Diaptomus* cf. *serbicus* collected in November 2017 in Limni Mourouza (GR173) and Limni Mourouzos (GR174) are available. This taxon is morphologically close to *Diaptomus serbicus*, a species reported from the Balkans to Italy (Kiefer 1978, Marrone et al. 2017), which in Greece is reported to occur only in the island of Kerkyra (Stephanides 1948, Zarfdjian and Economidis 1989).

3. Ostracoda

Among the collected ostracods, the cypridid *Potamocypris unicaudata* is new for the fauna of Greece. *Heterocypris incongruens* and *Tonnacypris lutaria*, two species typical of temporary ponds, dominated the ostracod assemblages of the 3170* sites only; conversely, the more euryecious *Cyclocypris ovum* was found only in the “non-3170*” ponds.

Notes on the assemblages

The collected crustaceans allowed us to determine four different assemblages, each one linked to a different habitat type (Figs. 3 and 4d). It is not surprising that the faunas occurring in the Anthili rice fields (GR164) and in the fish-inhabited brackish-water reservoir located at the mouth of the Spercheios River (GR171) are well-characterized and distinct from one another, and from those inhabiting the studied temporary ponds. Conversely, the sharp segregation of the crustacean assemblages occurring in the 3170* temporary ponds from those occurring in neighbouring, “non-3170*” temporary water bodies, was not obvious. Such a segregation has to be ascribed to the dominant presence of taxa linked to strictly temporary water bodies in the “3170* ponds” (i.e. *Chirocephalus diaphanus* s.l., *Daphnia atkinsoni*, *D. chevreuxi*, *Arctodiaptomus alpinus*, *Diaptomus* cf. *serbicus*, *Metacyclops minutus*, *Tonnacypris lutaria*), which are replaced by more euryecious taxa in the “non-3170*” sampled ponds (e.g., *Chydorus sphaericus*, *Arctodiaptomus pectinicornis*, *Acanthocyclops einslei*, *Cyclocypris ovum*). Among the 3170* ponds, Limni Nevropolis showed a mixed fauna, including both temporary pond-dwellers (e.g., *Chirocephalus diaphanus* s.l.) and more euryecious taxa (e.g., *Simocephalus vetulus*, *Chydorus sphaericus*). This is due to the astatic nature of Limni Nevropolis, which is fed both by rainfall surface run-off and by a spring.

CONCLUSIONS

The data collected so far from the two Sites of Community Interest (SIC) present in the Fthiotida area suggest the occurrence of different crustacean assemblages in "3170*" *vs*. "non-3170*" temporary ponds. This finding stresses the peculiarity of the biota of Mediterranean temporary ponds as defined by the EU "Habitats Directive" and, at the same time, highlights that an integrative analysis of different biological communities, using datasets from different taxonomic groups, is desirable to ensure effective choices for the long-term conservation of the whole biological diversity of a given area (Bagella et al. 2011, Guareschi et al. 2015). Accordingly, precautionary management efforts should be carried out in order to preserve these important water bodies. The current focus on the priority habitat "Mediterranean Temporary Ponds" grants an "umbrella protection effect" to their peculiar crustacean assemblages, whereas those species recorded only (or mostly) in the other temporary ponds should be the object of different, albeit less stringent, management measures. As highlighted by the high incidence of species recorded from single sites, the currently available data probably do not allow getting an exhaustive picture of the diversity of the crustacean fauna in the study area, and thus, there is a time-sensitive need for other surveys for a deeper understanding of these fragile ecosystems.

ACKNOWLEDGEMENTS

P. Delipetrou (National and Kapodistrian University, Athens) and I. Dimitriadis (National and Kapodistrian University, Athens) are warmly acknowledged for the support they provided in the planning and carrying out of the sampling activities as well as Anna N. Chapman (National and Kapodistrian University, Athens) for helping out during the sampling and curation of the specimens. V. Pieri (Piacenza, Italy) kindly identified the ostracods.

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Submitted: 27 May 2019

First decision: 4 July 2019

Accepted: 26 July 2019

Edited by Nico Cellinese

Appendix 1. Environmental variables registered in the studied water bodies. Temp: water temperature (°C); Cond: electrical conductivity (µS/cm); Turb: water turbidity; Macr: presence of aquatic macrophytes (see text for details).

Sites	**Habitat 3170***	**Code**	**Date**	**Temp**	**Cond**	**Turb**	**Macr**
Pond 1 Anopia	NO	GR160	28/05/2017	14.4	460	1	2.5
Limni Nevropolis	YES	GR161	05/05/2017	nd	nd	nd	nd
Limni Nevropolis	YES	GR161	28/05/2017	14.8	260	2	2
Nevropolis pool	NO	GR162	28/05/2017	12	140	3	1
Limni Louka	YES	GR163	05/05/2017	nd	nd	nd	nd
Limni Louka	YES	GR163	29/05/2017	15	68	1	1.5
Anthili rice fields	NO	GR164	29/05/2017	nd	nd	3	1
Pond 1 Katavothra	NO	GR165	30/05/2017	12	84	1.5	2
Artificial pond on the path to Livadies	NO	GR166	30/05/2017	10	39	1	2
Limni Greveno	YES	GR167	19/05/2017	nd	nd	nd	nd
Limni Greveno	YES	GR167	30/05/2017	19	37	1	1.5
Limni Alikaina	YES	GR168	19/05/2017	nd	nd	nd	nd
Limni Alikaina	YES	GR168	30/05/2017	22	24	1	1
Limni Livadies	YES	GR169	19/05/2017	nd	nd	nd	nd
Limni Livadies	YES	GR169	30/05/2017	23	18	1	2
Limni Livadies	YES	GR169	23/11/2017	nd	nd	nd	nd
Pond at the mouth of Spercheios river	NO	GR170	31/05/2017	nd	nd	1	1
Reservoir at the mouth of the Spercheios river	NO	GR171	31/05/2017	23	2700	2	2
Pond close to the village of Kastriotissa	NO	GR172	31/05/2017	22	670	1	1.5
Mourouza	YES	GR173	23/11/2017	nd	nd	nd	nd
Mourouzos	YES	GR174	23/11/2017	nd	nd	nd	nd
Mourouzos	YES	GR174	lab culture	nd	nd	nd	nd